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TITLE: Tea-derived feed additive and animal feed containing the same

## BSPR:

(1) Tannins, such as (+)-catechin, (+)-gallocatechin, (-)-gallocatechin gallate, (-)-epicatechin, (-)-epicatechin gallate, (-)-epigallocatechin, (-)-epigallocatechin gallate, free teaflavin, teaflavin monogallate A, teaflavin monogallate B and teaflavin digallate, all of which are polyphenol compounds contained in tea extract, are effective against noninfectious diarrhea, when used in feed; specific antibodies against pathogenic bacteria or viruses, or specific antibodies against toxins produced thereby, exhibit better effect against infectious diarrhea, when used in feed together with said polyphenol compounds, in comparison with said polyphenol compounds used alone.

## BSPR:

For inhibitory effect against infectious diarrhea, the dose of the feed additive in combination with a specific antibody is normally 0.3 to 25 mg/kg body weight, preferably 2 to 15 mg/kg body weight, as daily dose of polyphenol compounds. If the dose is less than 0.3 mg/kg body weight, no suppressive effect against infectious diarrhea is obtained. Polyphenol compounds can be quantified by the amount of tannin determined by the officially approved method for tannin analysis [Chakenhou, Vol. 71, pp. 43-74 (1990)].

## BSPV:

(11) The animal feed as described in the above (8), which further comprises a specific antibody against infectious microorganism or virus, or toxins produced thereby;

## BSPV:

(12) The animal feed as described in the above (11), wherein the specific antibody is an egg yolk antibody obtained from eggs of egg laying hens hyperimmunized with infectious microorganism or virus, or a toxin produced thereby;

## BSPV:

(13) The animal feed as described in the above (11), wherein the specific antibody is a milk antibody obtained from milk of a mammal hyperimmunized with infectious microorganism or virus, or a toxin produced thereby;

## BSPV:

(14) The animal feed as described in the above (11), wherein the feed being formulated so that not less than 1 mg/kg of body weight of the specific antibody is given to animals, the specific antibody having a titer of not less than 1.5 times as high as that of a blank in an enzyme immunoassay;

## DEPR:

Each of the following causative bacteria for bovine infectious diarrhea was grown in a brain heart infusion medium: Salmonella dublin, Escherichia coli 0-88, Escherichia coli 0-99 and Escherichia coli 987P. Also, each of bovine Rotavirus KK-3 and bovine Rotavirus NCDV was cultured using MA 104 cells of rhesus origin as the host cells grown in Eagle's MEM medium. Egg-laying hens were hyperimmunized using each of the bacteria and viruses as the antigen. From 10 kg of the egg yolk of the hens, the egg yolk antibody specific to each bacterium was obtained in an amount of 45 g.

## DEPR:

From Tables 6 and 7, it is found that the calves in Group I showed low fecal scores and decreased counts of Salmonella dublin in feces as compared with the calves in Groups G, H, and H2. This indicates that the tea extract of the present invention effectively inhibits infectious diarrhea. Similar results were obtained when the same procedures as this experiment were followed using the tea extract prepared in Example 2 or 3. Moreover, when the same type of experiment as this was carried out using extract from black tea or oolong tea, similar effects were obtained. When a similar experiment on infectious diarrhea was conducted in animals infected with Escherichia coli 0-88, Escherichia coli 0-99, Escherichia coli 987P, bovine Rotavirus KK-3 or bovine Rotavirus NCDV, similar results were obtained.

## DEPR:

Fifteen cows that had been infected with Salmonella dublin were divided into the following 3 groups of 5 cows each and fed for 5 weeks: Group J where only the basic feed as listed in Table 3 was given; Group K where 7.5 g/day of the tea extract prepared in Example 1 was given; Group L where 7.5 g/day of the tea extract prepared in Example 1 and 6 g/day of the specific egg yolk antibody prepared in Example 4 were given; Group K2 where 6 g/day of the specific egg yolk antibody prepared in Example 4 was given. The tea extract and the specific egg yolk antibody were mixed with the basic feed as listed in Table 3 and given to the animals. The animals were allowed to have access to water ad libitum. Similarly to Experimental Example 3 as indexes for diarrhea, fecal score and Salmonella dublin count in feces were determined from week 0 to week 5 after the start of the experiment. The results are shown in Tables 8 and 9.

## DEPR:

From Tables 8 and 9, it is found that the cows in Group L show lower fecal scores and smaller counts of Salmonella dublin in feces as compared with the cows in Groups J, K, and K2. This indicates that the tea extract of the present invention effectively inhibits infectious diarrhea. Similar results were

obtained when the same procedures as this experiment were followed using the tea extract prepared in Example 2 or 3. Moreover, when the same type of experiment as this was carried out using extract from black tea or oolong tea, similar results were obtained. When a similar experiment was conducted using Escherichia coli 0-88, Escherichia coli 0-99, Escherichia coli 987P, bovine Rotavirus KK-3 or bovine Rotavirus NCDV, similar results were obtained.

DEPR:

From Tables 22 and 23, it is found that the calves in Group I show lower fecal scores and decreased bacterial counts of Salmonella dublin in feces as compared with the calves in Groups G and H. This indicates that (-)-epigallocatechin gallate of the present invention effectively inhibits infectious diarrhea. When similar experiment on infectious diarrhea was conducted in animals infected with Escherichia coli 0-88, Escherichia coli 0-99, Escherichia coli 987P, bovine Rotavirus KK-3 and bovine Rotavirus NCDV, similar results were obtained.

DEPR:

Fifteen cows infected with Salmonella dublin were divided into the following 3 groups of 5 cows each and fed for 5 weeks: Group J where only the basic feed listed in Table 3 was given; Group K where 500 mg/day of (-)-epigallocatechin gallate prepared in Example 5 was given; and Group L where 500 mg/day of (-)-epigallocatechin gallate prepared in Example 5 and 6 g/day of the specific egg yolk antibody prepared in Example 4 were given. The (-)-epigallocatechin gallate or the specific egg yolk antibody were mixed with the basic feed listed in Table 3 and given to the animals. The animals were allowed to have access to water ad libitum. As indexes for diarrhea, fecal score and Salmonella dublin count in feces were measured from week 0 to week 5 after the start of the experiment. The results are shown in Tables 24 and 25.

DEPR:

From Tables 24 and 25, it is found that the cows in Group L gave low fecal scores and decreased counts of Salmonella dublin in feces as compared with the cows in Groups J, K, and K2. This indicates that (-)-epigallocatechin gallate of the present invention effectively inhibits infectious diarrhea. When a similar experiment was conducted in animals infected with Escherichia coli 0-88, Escherichia coli 0-99, Escherichia coli 987P, bovine Rotavirus KK-3 or bovine Rotavirus NCDV, similar results were obtained.